Regulation of the Epstein-Barr virus C promoter by the *OriP*-EBNA1 complex

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Göteborgs universitet kommer att offentligen försvaras i Sahlgrenska sjukhusets aula,
fredagen den 31:e oktober 2008, kl. 9:00

av

Cecilia Boreström

Opponent: Dr. Bettina Kempkes, Helmotz Zentrum, München, Tyskland

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Avhandlingen baseras på följande arbeten:

I. **Functional Interaction of Nuclear Factor Y and Sp1 is Required for Activation of the Epstein-Barr virus C promoter.**
Boreström, C., Zetterberg, H., Liff, K., and Rymo, L.
Journal of Virology (2003) 77(2): p. 821-9

II. **Multiple EBNA1-binding sites within *oriPI* are required for EBNA1-dependent transactivation of the Epstein-Barr virus C promoter.**
Zetterberg, H., **Boreström, C.**, Nilsson, T., and Rymo, L.
International Journal of Oncology (2004) 25 (3): p 693-6

III. **Functional Interaction of Oct transcription factors with the Family of Repeats in Epstein-Barr virus *oriP*.**
Almqvist, J., Zou, J., Linderson, Y., **Boreström, C.**, Altiok, E., Zetterberg, H., Rymo, L., Pettersson, S., and Ernberg, I.
Journal of General Virology (2005) 86(5): p. 1261-7

IV. **Bright, E2F1 and Oct-2 bind the Epstein-Barr virus C promoter and the *oriPI*, linking the promoter to the enhancer**
Boreström, C., Rüetschi, U., and Rymo, L.
In manuscript (2008)

Handledare: Professor Lars Rymo, Institutionen för Biomedicin

GÖTEBORGS UNIVERSITET

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Cecilia Boreström
Institute of Biomedicine, University of Gothenburg, Sweden

Epstein-Barr Virus (EBV) is an exclusively human, lymphotropic herpes virus that infects more than 90% of the population worldwide. Primary infection usually occurs during the early years of life and does not result in any recognized disease. EBV is the causative agent of infectious mononucleosis and is associated with various malignancies including Burkitt's lymphoma (BL), Hodgkin's disease (HD), and nasopharyngeal carcinoma (NPC). In most immunocompetent individuals the virus is, however, harbored for life within latently infected resting memory B cells, causing no symptoms. *In vitro*, EBV efficiently transforms resting B cells to activated lymphoblasts that express a repertoire of viral antigens (EBNA1-6 and LMP1), all of which have been directly implicated in the immortalization process. Immediately post-infection, EBNA2 and -5 are expressed from the W promoter (Wp). Within 36 hours there is a switch in promoter usage from Wp to the upstream C promoter (Cp). Transcription from Cp leads to expression of all EBNAs from a polycistronic transcription unit that is spliced to yield the different EBNA proteins. EBNA1 forms multiple homodimers that bind to a portion of the latency origin of replication (*oriPI*) that functions as an EBNA1-dependent enhancer of Cp. The mechanism for the interaction between the *oriPI*-EBNA1 complex and the Cp is not completely understood at the molecular level. The aim of this thesis was to identify and characterize novel interaction partners in this macromolecular complex.

The interactions of the transcription factors NF-Y and Sp1 with the Cp were previously established in our lab. In paper I we studied these interactions further using transient transfections, establishing that NF-Y and Sp1 co-stimulate Cp and that the *oriPI*-EBNA1-induced transactivation of Cp requires concomitant expression of both proteins. Furthermore, using the lymphoblastoid cell line EREB2-5, in which EBNA2 function is regulated by estrogen, we demonstrated that inactivation of EBNA2 resulted in decreased expression of NF-Y and down-regulation of Cp. Knowing that resting B cells do not express NF-Y and observing that this factor is essential for Cp activation, we suggest that its up-regulation post-infection may contribute to the Wp-to-Cp switch in primary EBV infection. The *oriPI* contains 20 repeats of the EBNA1 binding domain. In paper II we used a series of *oriPI*-deletions in *oriPI*-CpCAT reporter plasmids in transient transfections, to determine the number of EBNA1 binding repeats necessary for efficient transactivation of the Cp. We showed that eight or more repeats are necessary for this effect, which underscores the complexity of the transactivation process. In papers III and IV we set out to identify novel interaction partners of the *oriPI*- and -170Cp regions using EMSA and DNA affinity purification coupled with mass spectrometry. Three novel protein interactions with the *oriPI* and the Cp were identified. The transcription factors Bright, E2F1 and Oct-2 were found to bind both sequences *in vitro* and *in vivo*, possibly linking the *oriPI* and the Cp. The binding sites of all three proteins were mapped to a short segment of Cp that is essential for both *oriPI*-dependent and -independent transcriptional activation, indicating that the interactions are important for Cp activity. In transient transfections, we demonstrated that exogenous Oct-2 or Bright expression up-regulated *oriPI*-dependent Cp activation in the absence of EBNA1. Finally, endogenous Bright expression was shown to correlate with latency III but not latency I and II expression patterns in EBV positive cell lines, further supporting the notion that Bright expression is important for Cp transcriptional activity *in vivo*.

Keywords: *Epstein-Barr virus, EBNA1, NF-Y, Sp1, Oct-2, Bright, E2F1, transcriptional regulation*

ISBN 978-91-628-7557-2